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ABSTRACT

Validation of the Bovine Corneal Opacity and Permeability Test with Surfactants

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Previous evaluations of the Bovine Corneal Opacity and Permeability Assay (BCOP) demonstrated correlation with the Draize Eye Irritation Evaluation performed in rabbits. In this validation study, we evaluated the results of the BCOP relative to an abbreviated 3 rabbit Draize eye test using surfactant dilutions. The surfactants chosen for analysis were sodium lauryl sulfate, benzalkonium chloride, cetylpyridinium chloride, polyoxyethylene 9 lauryl ether, dioctyl sulfosuccinate and Tween 80. The dilutions for each surfactant were chosen based on their ability to produce a range of responses from very low to moderate ocular irritation in rabbits. Severely irritating concentrations were not selected in order to insure that no unnecessary pain was inflicted on the rabbits. The calculated *in vitro* scores from the BCOP assay were compared with the day 1 weighted Draize mean scores for the 3 animal rabbit eye irritation evaluations.

BCOP *in vitro* scores ranged from less than 0 to approximately 30. Day 1 Draize mean scores ranged from 0 to greater than 50. The results indicated that increases in *in vitro* scores using the BCOP were associated with increasing Draize scores for benzalkonium chloride, cetylpyridinium chloride, and polyoxyethylene 9 lauryl ether concentrations. An inverse relationship was noted for high concentrations of sodium lauryl sulfate and dioctyl sulfosuccinate. There was some indication that the opacity and permeability scores may be used independently to predict ocular irritation.

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INTRODUCTION

The Bovine Corneal Opacity and Permeability Test (BCOP) has been evaluated as a screening tool for the prediction of ocular irritation.[1, 2, 3, 4] The information supplied with the opacitometer used for the assay[5] suggested a broad classification scheme which categorized materials based on the *in vitro* scores as follows:

***IN VITRO* SCORES**	**CLASSIFICATION**
0 to 25	Mild Irritant
25.1 to 55	Moderate Irritant
55.1 and greater	Severe Irritant

MB Research has been validating the BCOP method relative to an abbreviated 3 rabbit Draize eye irritation test with *in vitro* scores being compared with day 1 Draize mean scores. Previous evaluations indicated that cosmetics or alcohols which produced an *in vitro* score of less than 10 were not Draize irritants, and shampoos which produced an *in vitro* score of less than 2 were not Draize irritants.[3]

The objectives of this study were to compare the results of a variety of surfactants in the BCOP assay with the results in the Draize eye irritation test, and to determine the BCOP *in vitro* scores which correspond to non-irritating Draize scores.

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TEST MATERIALS

Initially, six surfactants were selected for this evaluation, i.e., 2 anionic, 2 cationic and 2 non-ionic. The samples were purchased from Sigma Chemical Corp. The two anionic surfactants were sodium lauryl sulfate and dioctyl sulfosuccinate. The two cationic surfactants were benzalkonium chloride and cetylpyridinium chloride. The two nonionic surfactants were polyoxyethylene 9 lauryl ether and Tween 80 (polyoxyethylene sorbitan monooleate).
An additional anionic surfactant, Niaproof Type 8, was added to the study when the response of the other two anionic surfactants were found to differ from the expected relationship, i.e., high *in vitro* scores corresponding to high Draize scores. Distilled water was the diluent used for all Draize and BCOP studies.

DRAIZE METHOD

Three healthy New Zealand white rabbits, free from evidence of ocular irritation and corneal abnormalities, were dosed with each surfactant dilution. A dose of 0.1 ml was placed by syringe into the conjunctival sac of one eye of each animal after gently pulling the lower eyelid away from the eye. After instillation, the lids were held together for approximately 1 second to insure adequate distribution of the test article.

Each treated eye was examined for irritation of the cornea, iris and conjunctiva on days 1, 2 and 3 following dosing. Ocular reactions were graded according to the numerical Draize technique (Table 1)[6]. Additional signs were described.

The primary eye irritation score for each rabbit was calculated from the weighted Draize scale (Table 1) and the Mean Total Score (MTS) for each day was determined by averaging the individual primary eye irritation scores.

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BCOP METHOD

The bovine eyes were received from a local supplier and transported to MB Research Laboratories in Hanks Balanced Salt Solution in a refrigerated container. The eyes were examined within one hour after receipt and any cornea exhibiting evidence of vascularization, pigmentation, opacity or scratches was discarded.

Corneas which were free of defects were dissected from the surrounding tissues. A 2-3 mm rim of sclera was left attached to each cornea. The dissected corneas were mounted in specially designed holders segmented into anterior and posterior chambers which were filled separately. Each cornea was mounted allowing the epithelium of the cornea to project into the anterior chamber. The posterior chamber was filled with Minimal Essential Media supplemented with 1% Fetal Bovine Serum (MEM). The anterior chamber was then filled with MEM. Each cornea was visually inspected again to insure that there were no defects. The entire holder with the cornea was submerged in a 32°C water bath and allowed to equilibrate for at least one hour, but not longer than 2 hours.

Following equilibration, the holders containing the corneas were removed from the water baths. The MEM was removed from both chambers and the chambers refilled with fresh MEM. At this time, five corneas were selected for dosing with the test material and two were selected as controls. Measurements of opacity through the cornea were made using an OP-KIT™ opacitometer produced by Electro-Design Corporation of Rion, France. At each interval, each treated cornea was scored and compared to the two control corneas. A pre-exposure determination of opacity was made for each control by measuring against blanks supplied with the opacitometer. A pre-exposure determination of opacity was made for each of the 5 test corneas by comparing to each control cornea (a total of 10 determinations).

Following the pretest observations, the MEM was removed from the anterior chamber and a volume of 0.75 ml of the surfactant was applied to the epithelium of each of the five treated corneas. The holders and corneas were then placed in the 32°C water bath in a horizontal position to insure contact of the test material with the cornea. After 10 ±1 minute, the test substance (or MEM in the controls) was removed from the epithelium of the cornea and the anterior chamber by washing with MEM. All holders were then refilled with fresh MEM. A measurement of opacity was taken comparing each of the five treated corneas to the two control corneas. The corneas and holders were then returned to the water bath and incubated at 32°C for an additional two hours. At the end of the two hour period, the MEM was changed again and a measurement of opacity taken comparing each of the five treated corneas to the two control corneas. Immediately following this measurement, the MEM was changed in the posterior chamber of both the control and test corneas. The MEM was removed from the anterior chamber and replaced with 1.0 ml of 0.4% sodium fluorescein solution in both the treated and control corneas. Fresh holders and corneas were then returned to the 32°C water bath in a horizontal position to insure contact of the fluorescein with the cornea.

After 90 minutes, the fluid from the posterior chamber was removed and the amount of dye which had passed through the cornea was recorded as the optical density at 450 nm using a Spectronic 20 Spectrophotometer.

The corrected mean opacity score was calculated using the control and treated cornea opacity values as determined from the OP-KIT. The corrected mean optical density score was calculated using the control and treated optical density values from the fluorescein permeability analysis. The *in vitro* score was calculated as:

Corrected Mean Opacity Score[a] + 15 (Corrected Mean Optical Density Score).

[a] = Either the ten minute or 2 hour score, whichever is larger.

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RESULTS

The BCOP *in vitro* scores and corresponding day 1 Draize Mean Total Scores are presented in Table 2. The opacity score and optical density score, the two components of the *in vitro* score, are also included in Table 2. The results of the Draize ocular testing were also classified for levels of irritancy according to a modification of the original Draize interpretation using only 3 animals as follows:

Non-irritant	0 rabbit with positive scores
Indeterminate	1 rabbit with positive scores
Irritant	2 - 3 rabbits with positive scores

The day 1 Draize Mean Total Scores ranged from 0 to 24.33. The BCOP *in vitro* scores ranged from 2.56 to 38.10. Because of the odd response noted in the *in vitro* scores for the sodium lauryl sulfate, the 5.0, 10 and 20% concentrations were repeated. The result of the repeated concentrations are presented in Table 3.

DISCUSSION

For cationic and non-ionic surfactants, it appears a BCOP *in vitro* score of less than 10 corresponds to a non-irritating classification in the Draize rabbit eye test. However, the responses noted with anionic surfactants produced some equivocal results. The BCOP *in vitro* scores corresponding to non-irritant in the Draize tests were approximately 20 for the sodium lauryl sulfate and dioctyl sulfo-succinate. When the surfactant solutions were made more concentrated, the BCOP *in vitro* scores declined even though the Draize scores increased. The results using the Niaproof Type 8 were similar although not as pronounced.

Other materials tested in the past two years have produced similar equivocal results, but in most cases debris was noted in the anterior chamber of the BCOP holders during the 2 hour incubation period. It has been suggested that the test materials caused sloughing of the cells of the corneal epithelium which allowed additional light transmission with correspondingly lower opacity scores. However, no evidence of sloughing was found in the MEM of the anterior chamber during this study.

Gautheron, et al.[1], reported similar results with sodium lauryl sulfate and suggested that test materials which produced destruction of the corneal epithelium be classified as hazardous and suggested the permeability measurement as the endpoint of choice for assays in which the corneal epithelium is destroyed. In our studies, visual analyses of the cornea by the technician performing the study can normally confirm the presence of opacity, particularly when scores are >20. There was, however, no visual evidence of opacity in the corneas exposed to high concentrations of the anionic surfactants. There is some evidence that the permeability scores alone without the opacity scores may be a more valid indication of ocular damage when high concentrations of anionic surfactants are present since the permeability scores generally increased with increases in Draize scores.

This study suggests the use of the BCOP method is a valid predictor of ocular irritation for both cationic and non-ionic surfactant solutions and for dilute solutions of anionic surfactants. For more concentrated anionic solutions, it may be more appropriate to utilize the permeability part of the assay without the opacity part.

Future validation studies will be performed on mixtures of anionic and nonionic surfactants and cationic and nonionic surfactants. Histopathologic examination of the corneas following exposure to anionic surfactants is also being considered for future validation programs.

SCALE FOR SCORING OCULAR LESIONS[1]

(1) CORNEA:

(A)	Opacity: Degree of density (area most dense taken for reading):		
	No ulceration or opacity		0
	Scattered or diffuse areas of opacity (other than slight dulling of normal luster), details of iris clearly visible		1[2]
	Easily discernible translucent area, details of iris slightly obscured		2[2]
	Opalescent areas, no details or iris visible, size of pupil barely discernible		3[2]
	Opaque cornea, iris not discernible through the opacity		4[2]
(B)	Area of cornea involved:		
	One quarter (or less) but not zero		1
	Greater than one-quarter, but less than one-half		2
	Greater than one-half, but less than three-quarters		3
	Greater than three quarters up to whole area		4
	SCORE EQUALS A x B x 5	**Maximum Total**	**80**

(2) IRIS:

(A)	Values:		
	Normal		0
	Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive)		1[2]
	No reaction to light, hemorrhage, gross destruction (any or all of these)		2[2]
	SCORE EQUALS A x 5	**Maximum Total**	**10**

(3) CONJUNCTIVAE:

(A)	REDNESS (refers to palpebral and bulbar conjunctivae excluding cornea & iris):		
	Blood vessels normal		0
	Some blood vessels definitely hyperemic (injected)		1
	More diffuse, deeper crimson red, individual vessels not easily discernible		2[2]
	Diffuse beefy red		3[2]
(B)	CHEMOSIS		
	No swelling		0
	Any swelling above normal (includes nictitating membranes)		1
	Obvious swelling with partial eversion of lids		2[2]
	Swelling with lids about half closed		3[2]
	Swelling with lids more than half closed		4[2]
(C)	DISCHARGE		
	No Discharge		0
	Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)		1
	Discharge with moistening of the lids and hairs just adjacent to lids		2
	Discharge with moistening of the lids and hairs and considerable area around the eye		3
	SCORE EQUALS (A+B+C)x2	**Maximum Total**	**20**

The maximum total score is the sum of all scores obtained for the cornea, iris and conjunctivae.

[1] **Draize, J. H. et al. J. Pharm. Exp. Ther. 82:377-390, 1944.**

[2] **Indicates a positive response**

BCOP *IN VITRO* and DRAIZE SCORES FOR SURFACTANT DILUTIONS

TYPE	SURFACTANT	AQUEOUS DILUTION %	DAY 1 DRAIZE MTS	DRAIZE CATEGORY	BCOP *IN VITRO* SCORE[a]	CORRECTED MEAN OPACITY SCORE	CORRECTED MEAN OD SCORE
ANIONIC	SODIUM LAURYL SULFATE	1.0	2.67	-	-2.56	-3.1	0.036
		5.0	4.0	i	27.04	19.9	0.476
		10	14.33	+	16.17	7.2	0.598
		20	14.33	+	10.35	2.1	0.550
	DIOCTYL SULFOSUCCINATE	0.05	0	-	-0.67	-3.1	0.162
		0.10	0	-	8.29	7.6	0.046
		0.50	0	-	19.93	18.1	0.122
		1.0	0.67	-	17.09	12.8	0.286
		2.0	5.33	i	28.88	23.5	0.392
		5.0	13.67	+	12.47	10.0	0.166
		10	24.33	+	11.22	7.2	0.268
	NIAPROOF TYPE 8	1.0	0	-	0.06	0.3	-0.016
		5.0	0.67	-	22.1	6.8	1.02
		10	10.67	+	31.25	10.7	1.37
		20	24.33	+	34.95	13.2	1.45
		30	22.33	+	26.35	4.0	1.49

KEY: - = negative
+ = positive
i = indeterminate
[a]BCOP *In Vitro* Scores = Corrected Mean Opacity Score + 15 (Corrected Mean Optical Density Score)

BCOP *IN VITRO* and DRAIZE SCORES FOR SURFACTANT DILUTIONS

TYPE	SURFACTANT	AQUEOUS DILUTION %	DAY 1 DRAIZE MTS	DRAIZE CATEGORY	BCOP *IN VITRO* SCORE[a]	CORRECTED MEAN OPACITY SCORE	CORRECTED MEAN OD SCORE
NON- IONIC	POLYOXYETHYLENE 9 LAURYL ETHER	0.25	0	-	2.56	2.3	0.0175
		0.50	1.33	-	5.93	5.5	0.0285
		1.0	4.67	i	11.54	8.6	0.196
		5.0	7.33	+	26.75	6.2	1.37
		10	11.33	+	29.05	8.2	1.39
	TWEEN 80	20	0	-	1.23	0.002	1.2
		50	0	-	-0.18	-0.6	0.028
CATIONIC	BENZALKONIUM CHLORIDE	0.1	2.00	-	4.02	3.9	0.008
		0.2	9.33	+	10.86	9.6	0.084
		0.3	15.33	+	23.97	15.9	0.538
		0.5	16.00	+	36.12	28.0	0.5415
	CETYLPYRIDINIUM CHLORIDE	0.1	0.33	-	7.81	5.8	0.134
		0.3	4.67	+	18.56	14.3	0.280
		0.5	12.33	+	30.13	22.7	0.495
		1.0	16.67	+	37.12	27.2	0.628
		2.0	16.67	+	39.2	18.5	1.38

KEY: - = negative
+ = positive
i = indeterminate
[a]BCOP *In Vitro* Scores = Corrected Mean Opacity Score + 15 (Corrected Mean Optical Density Score)

IN VITRO SCORES FOR SODIUM LAURYL SULFATE DILUTIONS

AQUEOUS DILUTION	INITIAL *IN VITRO* SCORE[a]	REPEAT *IN VITRO* SCORE[a]	INITIAL CORRECTED MEAN OPACITY SCORE	REPEAT CORRECTED MEAN OPACITY SCORE	INITIAL CORRECTED MEAN OD SCORE	REPEAT CORRECTED MEAN O.D. SCORE
5.0	27.04	22.84	19.9	14.6	0.476	0.549
10	16.17	19.60	7.2	9.2	0.598	0.693
20	10.35	9.03	2.1	0.1	0.550	0.595

[a]BCOP *In Vitro* Scores = Corrected Mean Opacity Score + 15 (Corrected Mean Optical Density Score)

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